



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Michael J. Detmar et al.
 Serial No. : 09/536,087
 Filed : March 24, 2000
 Title : THROMBOSPONDIN-2 AND USES THEREOF

Art Unit : 1642
 Examiner : M. Yu

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Commissioner for Patents
 Washington, D.C. 20231

SECOND DECLARATION UNDER 37 C.F.R. §1.132 OF DR. MICHAEL DETMAR

I, Michael Detmar, a citizen of Germany, residing in Arlington, MA, hereby declare as follows:

1. I am Associate Professor of Dermatology at Harvard Medical School and Associate Biologist at Massachusetts General Hospital. I received my M.D. degree from the University of Freiburg Medical School, Germany, in 1984. After completion of my residency in dermatology at the Free University of Berlin, Germany in 1990, I held the position of Senior Dermatologist at the Dept. of Dermatology, Free University of Berlin, until 1993. In 1993, I joined the faculty of the Dept. of Dermatology, Harvard Medical School as a Visiting Assistant Professor of Pathology (1993-1996) and of Dermatology (1995-1997). In 1998, I joined the Dept. of Dermatology at Massachusetts General Hospital and was appointed Associate Professor of Dermatology, Harvard Medical School.
2. I am a co-inventor of the invention claimed in the above-identified patent application, and I have read and understand the contents of the present patent application.
3. I have also been advised and understand that the Examiner has rejected claims 1, 6, 7, 13-23, 53-61, 63-68 and 75-87 of the above-referenced application. The present claims are directed

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to methods of treating angiogenesis-dependent tumors by administering TSP-2 or specifically described fragments of TSP-2 having the ability to inhibit endothelial cell migration.

4. The work described in the specification (and additional work described in my previous declaration dated September 27, 2002), demonstrates that TSP-2 and TSP-2 fragments can inhibit microvascular endothelial cell migration *in vitro* and confirms that TSP-2 and TSP-2 fragments can treat angiogenesis-dependent tumors *in vivo*. This work is described in detail in the speciation, in my previous declaration, and in the reply submitted with this declaration, and is briefly summarized below.

5. Full-length TSP-2 protein significantly inhibited migration of human dermal microvascular endothelial cells *in vitro* (see page 39 of the specification, last paragraph). A fragment derived from a type I repeat of TSP-2 (containing amino acids 384-390 of TSP-2) also significantly inhibited migration of human dermal microvascular endothelial cells *in vitro* (see page 40 of specification). The ability of TSP-2 to inhibit tumor growth was confirmed *in vivo*. TSP-2 inhibited growth of squamous cell carcinoma cell grafts (A431 cells) and malignant melanoma cells (MeWo) in mice (see pages 40-42 of the specification). In my work described in my previous declaration dated September 27, 2002, an additional TSP-2 fragment covered by the claims was shown to inhibit angiogenesis of solid tumors *in vivo*. Namely, an N-terminal fragment of TSP-2, encoded by nucleotides 294-1883 of the TSP-2 coding sequence (containing the N-terminal procollagen domain of TSP-2, and the three type I repeats of TSP-2) inhibited growth of A431 tumors *in vivo*.

6. As discussed in the specification and shown by the work described above, the inhibitory effect of TSP-2 and active TSP-2 fragments on tumor growth *in vivo* correlates with the ability to inhibit migration of endothelial cells *in vitro*. In order to grow beyond minimal size, tumors (i.e., solid tumors) need to induce the growth of new blood vessels (angiogenesis) providing a lifeline for tumor sustenance and waste disposal. Angiogenesis is a multi-step process that can involve the following: the erosion of basement membrane by proteolytic enzymes; the migration of endothelial cells; and local proliferation of endothelial cells. One of ordinary skill in this field

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would understand that inhibiting any one of the steps necessary for angiogenesis to proceed normally would inhibit growth of any angiogenesis-dependent tumor. Thus, the ordinary skilled artisan would understand that the claimed methods can be useful in treating any tumor dependent on angiogenesis.

7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.



Michael Detmar, M.D.

7/31/2003

Date